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## LIPIDS OF SENESCENT LEAF TISSUE INDUCED BY INHIBITION OF SYNTHESIS AND ACCELERATION OF BREAKDOWN<sup>1</sup>

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**ABSTRACT.** Tissues were made senescent by inhibiting synthetic reactions with abscisic acid and by accelerating protein catabolism with L-serine in combination with kinetin. Squash (*Cucurbita maxima*) leaf discs were floated on the above-mentioned solutions, harvested, and the lipids extracted therefrom. Fatty acids of the acyl lipids were determined by gas chromatography, galactolipid concentrations were determined by sugar analyses, and phospholipid concentrations were determined by phosphorus analyses. All of the lipids were separated by thin-layer chromatography prior to analysis. Those tissues floated on abscisic acid or L-serine with kinetin contained less chlorophyll, less total fatty acids—especially linolenic acid, less glycolipid material, and less phospholipid material. Abscisic acid caused a decline in all of the cellular lipids as indicated by acyl group analysis and by analysis of the parental lipids. Possible effects of these compounds on chloroplast thylakoid stacking are discussed.

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### INTRODUCTION

The relative amounts of many biological compounds change in tissues that are undergoing senescence. Some of the macro-

molecules and other molecules decrease during this phase of development. The lipid profile of photosynthetic tissue is dependent upon the developmental state of the tissue (Newman et al. 1973). In this study we investigated the lipids of photosynthetic tissues that had been treated to either enhance protein breakdown or to in-

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hibit both DNA and RNA synthesis and consequently protein synthesis. It is assumed that ABA causes an inhibition of protein synthetic reactions as well as other effects (Thimann 1980) and that the application of L-serine with kinetin causes increased protein breakdown (Martin and Thimann 1972). The lipid profiles of the treated and nontreated tissues were then compared. Martin and Thimann (1972) suggested that L-serine is incorporated into the active center of a protease which in turn results in a vigorous promotion of senescence.

### METHODS AND MATERIALS

Blue Hubbard Squash (*Cucurbita maxima* Duchesne) was grown 5 wk under continuous light (13,000 lux; using Sylvania Cool White bulbs) at room temperature (ca 25 C). The first and second nodal leaves were surface sterilized with 10% Clorox; 1 cm leaf discs were removed. Forty leaf discs were placed on 25 ml of either water or on aqueous solutions of  $3.78 \times 10^{-5}$  M ABA,  $50 \times 10^{-3}$  M L-serine, or L-serine plus  $2.32 \times 10^{-5}$  M kinetin. The discs were incubated 6 days in continuous light. The discs then were boiled 3 min in chloroform-methanol (2:1, v/v) and homogenized in a ground-glass homogenizer. The sample was filtered, and a portion of the filtrate was removed for chlorophyll analysis. Three replications of each treatment were analyzed for the various components.

The chlorophyll analysis was done according to Arnon (1949). The chloroform-methanol was evaporated, and the sample was redissolved in 80% acetone. Absorbancies were read at 645 and 663 nm.

The remaining portion of each sample was washed 3 times with acidified water (2 drops 5 N  $\text{H}_2\text{SO}_4$  in 70 ml water). The chloroform phase was then dried with anhydrous  $\text{Na}_2\text{SO}_4$  and stored under  $\text{N}_2$  at 0 C.

A portion of the sample was used for two-dimensional thin-layer chromatography (Gurr and James 1971). The first dimension was developed in chloroform-methanol-7 N ammonia (65:25:4, v/v), and the second dimension was developed in chloroform-methanol-acetic acid-water (170:15:15:2, v/v). Lipophilic spots on the thin-layer plates were visualized with  $\text{I}_2$  vapors and identified by standards and by comparison with results found in the literature (Gurr and James 1971). The spots were eluted from the plates and analyzed for phosphorus and galactose content. The phosphorus analysis was done according to Marinetti (1962). The galactose analysis consisted of heating the sample at 100 C in 2 ml of 1 N  $\text{H}_2\text{SO}_4$  for 1 hr, then adding 12 ml of cold 98%  $\text{H}_2\text{SO}_4$ , and heating for an additional 5 min. The absorbance was measured at 322 nm.

A third portion of the sample was used for fatty acid analysis. The samples were transesterified with

methanolic-HCl (2.5% HCl, w/w) under reflux for 1.5 hr in an atmosphere of  $\text{N}_2$  (Kates 1964). The methyl esters were purified by passing the sample through silicic acid columns. The fatty acid methyl esters were resolved by gas chromatography on a 2.5-m column of 10% DEGS (diethylene glycol succinate) on acid-washed Chromosorb W. Separations were made isothermally at 185 C; the chromatograph contained a thermal conductivity detector.

### RESULTS AND DISCUSSION

From the time the leaf discs were removed to the time of harvest, the chlorophyll concentration in the leaf discs of those tissues floated on water alone decreased 47%. The changes in the chlorophyll content after floating on the various solutions for 6 days are given in table 1. Leaf tissue exposed to  $2.32 \times 10^{-5}$  M kinetin contained about the same amount of chlorophyll as that exposed to water alone (controls). In contrast, those tissues exposed to either ABA or to L-serine with kinetin contained much less chlorophyll. Serine alone (without added kinetin) does not cause the marked reduction in leaf chlorophylls (Martin and Thimann 1972). Using the chlorophyll content as an indication of the degree of senescence, both ABA and L-serine with kinetin accelerated the senescence process. When the tissue was harvested after a 6-day exposure period, the ABA-treated tissue was quite yellow (visually) but turgid; whereas the

TABLE 1

*Chlorophyll content of the treated and untreated samples.*

Treatment	Total chl	Chl a/b
	mg/40 leaf discs of 1 cm diameter	Ratio
Water control		
floated 6 days	$0.57 \pm 0.03^*$	2.50
$2.32 \times 10^{-5}$ M kinetin	$0.55 \pm 0.03$	2.50
$3.78 \times 10^{-5}$ M abscisic acid	$0.20 \pm 0.04$	2.46
$50 \times 10^{-3}$ M L-serine + $2.32 \times 10^{-5}$ M kinetin	$0.10 \pm 0.01$	1.96

\*  $\pm 1$  SD

TABLE 2.  
Fatty acid content in  $\mu$ moles per 40 leaf discs of the treated and untreated samples.

Treatment	Fatty acid						Total fatty acid
	16:0	16:1	18:0	18:1	18:2	18:3	18:3/16:0
Water control floated 6 days*	1.84 $\pm$ 0.08	0.24 $\pm$ 0.16	0.18 $\pm$ 0.05	0.21 $\pm$ 0.04	0.17 $\pm$ 0.04	12.2 $\pm$ 5.3	14.84 $\pm$ 7.24
2.32 $\times 10^{-5}$ M kinetin	1.42	0.12	0.13	0.06	Trace	5.77	7.65
3.78 $\times 10^{-5}$ M abscisic acid	0.51	0.23	0.08	0.00	Trace	1.05	2.56
50 $\times 10^{-5}$ M L-serine + 2.32 $\times 10^{-5}$ M kinetin	1.32	0.45	0.11	0.03	Trace	0.50	3.11
		0.55	0.11	0.17		0.12	0.71
							0.38

\* Average of 3 replications for all values

L-serine with kinetin-treated tissue seemed to be more flaccid than the other tissues.

From the time leaf discs were removed to the time of harvest, palmitic and linolenic fatty acids decreased 14% and 20%, respectively. The control tissue after 6 days floating contained the highest fatty acid content—about twice that of kinetin-treated (table 2). The total fatty acid content of the ABA-treated tissue and that of the L-serine with kinetin-treated tissue was much less than that of the controls or the tissue treated with kinetin alone. It is especially important to note the relative decline in C18:3 (linolenic acid) in the treated tissue, since senescent tissues often contain less C18:3 than do mature tissues (Newman et al. 1973). The ABA and L-serine with kinetin both caused a marked reduction in the C18:3 content. Since the relative content of C18:3 can be used as an index of the developmental state of the photosynthetic tissue, it may be postulated that both L-serine and ABA accelerated the development toward the senescent state. It could, therefore, be suggested that in this case senescence may result from inhibition of protein synthetic mechanisms (as affected by ABA) or from enhancement of catabolic activities (as affected by L-serine in combination with kinetin).

From the time the leaf discs were removed to the time of harvest monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) decreased 46% and 16%, respectively. The water control tissue floated for 6 days, and that treated with kinetin alone contained higher concentrations (than the treated tissues) of the 2 glycolipids and phosphatidyl glycerol (PG) lipids which are found in high concentrations in the chloroplast (table 3). However, those tissues treated with kinetin alone contained decreased amounts (than the controls) of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). PC is associated with membranes throughout the cell but can also be found in the chloroplast envelope (Poincelot 1973).

TABLE 3  
Lipid content in  $\mu\text{moles}/40$  leaf discs of the treated and untreated samples\*.

Treatment	Monogalactosyl diacylglycerol	Digalactosyl diacylglycerol	Phosphatidyl choline	Phosphatidyl glycerol	Phosphatidyl ethanolamine
Water control floated 6 days	$2.73 \pm 0.43$	$1.13 \pm 0.23$	$2.14 \pm 0.093$	$2.13 \pm \text{---}^{**}$	$2.14 \pm \text{---}$
$2.32 \times 10^{-5}$ M kinetin	$2.60 \pm 0.11$	$1.41 \pm 0.42$	$0.27 \pm 0.087$	$2.13 \pm \text{---}$	$0.25 \pm 0.034$
$3.78 \times 10^{-5}$ M abscisic acid	$0.55 \pm 0.02$	$0.25 \pm 0.18$	$0.014 \pm \text{---}$	$0.021 \pm 0.010$	$0.021 \pm 0.010$
$50 \times 10^{-3}$ M L-serine + $2.32 \times 10^{-5}$ M kinetin	$0.76 \pm 0.25$	$0.30 \pm 0.04$	$0.007 \pm \text{---}$	$0.18 \pm 0.17$	$0.062 \pm 0.050$

\*Three replications for all values except \*\*

\*\*Only 2 samples comprised this mean

There is very little PE in the chloroplast envelope and none in the chloroplast lamellae according to the above-mentioned report and none in the chloroplast envelope (Douce 1974). ABA and L-serine with kinetin markedly reduced the amounts of all the lipids (glycolipids and phospholipids both).

It could be suggested from these data that the membranous constitution of the treated, possibly senescent tissues, was markedly changed. Membrane integrity would, obviously, be of fundamental importance in maintaining the various functions of the cell, and therefore membrane changes must be concomitant with the senescence phenomenon.

Since L-serine caused a decrease in the chl a/b ratio, then L-serine probably caused a greater effect on the stroma thylakoids as opposed to the granal stacks. Ultrastructurally the chloroplast may show the first changes during leaf senescence. Further, within the chloroplast, often in senescence the stroma thylakoids may decline in amount with a concomitant increase in the granal stacking (Huber and Newman 1976). This would also be suggested by the changes in the chlorophyll a/b ratio. However, during the late stages of senescence in green tissues the granal thylakoids also disappear, even before visible changes in the chloroplast envelope (Tuquet and Newman 1980).

ABA did not have this differential effect, suggesting that the stroma and granal thylakoids were broken down at the same rate. Kinetin caused a decline in PE which is associated with non-chloroplastic membranes (Mudd 1980) but had no effect on MGDG, DGDG, and PG which are found in chloroplast membranes. This suggests that even though kinetin seems to preserve the chloroplast structure it may have affected a breakdown of non-chloroplast membranes. L-serine and ABA, however, affected both the chloroplastic and non-chloroplastic membranes. We suggest that L-serine enhanced protease throughout the cell. Consequently the total cellular membranes were decreased in amount.

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